R E M A R K S

As of the Preliminary Amendment filed on February 28, 2003, claims 1-14 were pending in the present application. Claims 2, 4, and 11 are canceled herein. Claims 3, 5, 6, and 13 are amended. Claims 15-27 are added. No new matter is inserted into the application.

Status of the Application

The Office Action dated February 25, 2003 presents the examination of claims 1-6, 10, and 11, which remain rejected. The mailing date of the Office Action precedes the date of the Preliminary Amendment filed on February 28, 2003. Thus, the Examiner did not consider the Preliminary Amendment in connection with the outstanding Office Action, as noted in the Communication from the Examiner dated March 14, 2003. In the Communication, the Examiner states that the Preliminary Amendment does not address all issues of record in the outstanding Office Action. Applicants respectfully submit that the instant Reply addresses all remaining issues of the Office Action and is therefore a complete response. Upon entry of this Reply, claims 1, 3, 5-10, and 12-27 should be pending.

Rejection under 35 U.S.C. § 112, first paragraph, new matter

The Examiner maintains the rejection of claim 1 under 35 U.S.C. § 112, first paragraph for allegedly containing new matter. Applicants respectfully traverse. Reconsideration of the claim and withdrawal of the instant rejection are respectfully requested.

The Examiner asserts that there is no support in the specification for a mutant α-amylase comprising an amino acid sequence which is at least 70% homologous to SEQ ID NO:1, as recited in claim 1. Applicants respectfully disagree. Support for a mutant α-amylase comprising an amino acid sequence which is at least 70% homologous to SEQ ID NO:1 is found throughout the specification, such as on page 3, lines 9-10 and 16-17, page 4, lines 23-25, and in the abstract. Furthermore, SEQ ID NO:2 is a liquefying alkaline α-amylase having 66.9% identity to SEQ ID NO:1. In this regard, the Examiner's attention is drawn to Exhibit 1 (attached hereto) which provides a sequence alignment between SEQ ID NO:1 and SEQ ID NO:2, using the matrix file BLOSUM50. SEQ ID NO:2 is referred to in the specification at least on page 7, line 21 to page 8, line 2.

For this reason, the rejection is improper. Withdrawal thereof is respectfully requested.

Rejection under 35 U.S.C. § 112, first paragraph, written description

The Examiner maintains the rejection of claims 1, 5 and 6 under 35 U.S.C. § 112, first paragraph for allegedly not being described in the specification. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Claim 1

The Examiner maintains that the claim 1 encompasses a protein variant having an untold number of mutations. Applicants respectfully disagree.

Claim 1 is directed to a mutant α-amylase obtained by making a substitution or deletion of at least one amino acid residue of specific positions in SEQ ID NO:1, or by making a substitution or deletion of at least one amino acid residue corresponding to the above-mentioned amino acid residue in a sequence having at least 70% homology to SEQ ID NO:1, wherein said at least one amino acid residue is selected from the group consisting of: the 11^{th} Tyr, 16^{th} Glu, 49^{th} Asn, 84^{th} Glu, 144^{th} Ser, 167^{th} Gln, 169^{th} Tyr, 178^{th} Ala, 188^{th} Glu, 190^{th} Asn, 205^{th} His and 209^{th} Gln, and said mutant α-amylase possesses increased heat resistance and maintains resistance to chelating agents when compared to SEQ ID NO:1, and said mutant α-amylase comprises an

amino acid sequence which is at least 70% homologous to SEQ ID NO:1. In other words, the mutant α-amylase must (1) be derived from an amino acid sequence corresponding to SEQ ID NO:1 or an amino acid sequence 70% homologous to SEQ ID NO:1, (2) have a substitution or deletion of the 11th Tyr, 16th Glu, 49th Asn, 84th Glu, 144th Ser, 167th Gln, 169th Tyr, 178th Ala, 188th Glu, 190th Asn, 205th His, or the 209th Gln, (3) possess the specific functions of increased heat resistance and resistance to chelating agents, and (4) have a resulting amino acid sequence which is at least 70% homologous to SEQ ID NO:1. Thus, the mutant α-amylase mutant is defined by several limitations in the claim.

The specification provides several examples of mutant α-amylases according to the present invention. As noted above, SEQ ID NO:2 is a liquefying alkaline α-amylase having 66.9% identity to SEQ ID NO:1 (see Exhibit 1). SEQ ID NO:4 is a liquefying alkaline α-amylase having 96.5% identity to SEQ ID NO:1. In this regard, the Examiner's attention is drawn to Exhibit 2 (attached hereto) which provides a sequence alignment between SEQ ID NO:1 and SEQ ID NO:4, using the matrix file BLOSUM50. SEQ ID NO:4 is referred to in the specification at least on page 5, lines 19-27. Other examples, although not listed by a particular SEQ ID, are described in Examples 5-10, pages 22-27 of the specification.

For the Examiner's convenience, the particular examples disclosed in the specification are listed in the following table:

MUTANT α-AMYLASE	SUPPORT IN SPECIFICATION
Y11F	Page 23
N49S	Page 23
E84Q	Page 23
S144P	Page 23
Q167E	Page 23
Y169K	Page 23
A178Q	Page 23
E188D	Page 23
N190F	Page 23
Q209V	Page 23
Q167E/Y169K	Page 24
N190F/Q209V	Page 24
Q167E/Y169K/N190F/Q209V	Page 24
S144P/N190F/Q209V	Page 25
E16P/S144P/N190F/Q209V	Page 25
M107L/Q167E/Y169K/N190F/Q209V	Page 26
N49S/M107L/Q167E/Y169K/N190F/Q209V	Page 26
N49S/M107L/H205R/Q167E/Y169K/N190F/Q209V	Page 26
LA-K38AMY	Pages 26-27
LA-K38AMY/Q167E/Y169K/N190F/Q209V	Page 27

These mutant α-amylases all have at least 95% sequence identity to SEQ ID NO:1.

As such, contrary to the Examiner's remarks, claim 1 does not encompass an unduly broad number of species. For this reason, the rejection is improper. Withdrawal thereof is respectfully requested.

Claims 5 and 6

Regarding claims 5 and 6, the Examiner appears to assert that the recitation of "two kinds of mutations" renders the

claims open to any number of mutations and represents an enormous genus of mutant α-amylases. In order to answer this rejection, claims 5 and 6 are amended to recite "a first mutation" and "a second mutation." Thus, the instant rejection is overcome.

Rejection under 35 U.S.C. § 112, first paragraph, enablement

Claims 1-6, 10, and 11

The Examiner rejects claims 1-6, 10, and 11 under 35 U.S.C. § 112, first paragraph, for allegedly not being enabled by the specification. Claims 2, 4, and 11 are canceled, thus rendering rejection thereof moot. Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

The Examiner maintains her assertion that the pending claims are not enabled by the specification. Specifically, the Examiner again asserts that claim 1 encompasses a protein variant having an untold number of mutations. Applicants respectfully disagree.

As noted above, the mutant α-amylase recited in claim 1 must (1) be derived from an amino acid sequence corresponding to SEQ ID NO:1 or an amino acid sequence 70% homologous to SEQ ID NO:1, (2) have a substitution or deletion of the 11^{th} Tyr, 16^{th} Glu, 49^{th} Asn, 84^{th} Glu, 144^{th} Ser, 167^{th} Gln, 169^{th} Tyr, 178^{th} Ala,

188th Glu, 190th Asn, 205th His, or the 209th Gln, (3) possess the specific functions of increased heat resistance and resistance to chelating agents, and (4) have a resulting amino acid sequence which is at least 70% homologous to SEQ ID NO:1. Thus, the mutant α-amylase mutant is defined by several limitations in the claim.

As such, contrary to the Examiner's remarks, the claims do not encompass an unduly broad number of species that are not enabled by the specification. For this reason, the rejection is improper. Withdrawal thereof is respectfully requested.

Rejection under 35 U.S.C. § 112, second paragraph

The Examiner rejects claims 1-6 and 10 under 35 U.S.C. § 112, second paragraph for allegedly being indefinite. Claims 2 and 4 are canceled, thus rendering rejection thereof moot. Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Claim 1

The Examiner asserts that claim 1 is unclear for reciting "at least one amino acid residue." Applicants respectfully disagree. Claim 1 is directed to a mutant α-amylase obtained by making a substitution or deletion of at least one amino acid

residue of specific positions in SEQ ID NO:1 or a homolog of SEQ ID NO:1. Applicants respectfully submit that it is clear from this language that at least one of the amino acid substitution/deletion must correspond to the 11th Tyr, 16th Glu, 49th Asn, 84th Glu, 144th Ser, 167th Gln, 169th Tyr, 178th Ala, 188th Glu, 190th Asn, 205th His, or the 209th Gln as noted in the claim. Thus, the rejection is improper and should be withdrawn.

Claims 2-4

The Examiner rejects claims 2-4 for reciting "liquefying α-amylase." Claims 2 and 4 are canceled. Regarding claim 3, the Examiner asserts the specification does not clearly define what is a "liquefying α-amylase." Claim 3, as amended, does not recite a "liquefying α-amylase." Thus, the instant rejection is overcome.

Claims 2 and 5

The Examiner rejects claims 2 and 5 for reciting "a substitution of a sequence…from the amino terminus…." Claim 2 is canceled. Regarding claim 5, the Examiner asserts that the exact position of the amino terminus is unclear. Applicants amend claim 5 to clarify that the second mutation consists of a substitution of a sequence corresponding to the 11th amino acid to the 100th amino acid from the N-terminal end of the sequence

(i.e., the first amino acid). Thus, the instant rejection is overcome.

Claim 5

The Examiner asserts that "a first (second) mutation is" in claim 5 is unclear. The phrase is amended to "a first (second) mutation consists of." Thus, the instant rejection is overcome.

Claim 6

Similarly, the Examiner asserts that "first (second) mutation comprises" in claim 6 is contradictory to claim 5, which recites "consists of." Claim 6 is amended to recite "a first (second) mutation consists of." Thus, the instant rejection is overcome.

Conclusion

Applicants respectfully submit that the above amendments and/or remarks fully address and overcome the rejections and objections of record. The instant claims are now in condition for allowance. Early and favorable action by the Examiner is respectfully requested.

If there are any minor matters precluding allowance of the application which may be resolved by a telephone discussion, the Examiner is respectfully requested to contact Kristi L. Rupert, Ph.D. (Reg. No. 45,702) at (703) 205-8000.

Pursuant to the provisions of 37 C.F.R. §§ 1.17 and 1.136(a), the Applicants hereby petition for an extension of two (2) months to July 25, 2003, in which to file a reply to the Office Action. The required fee of $410.00 is enclosed herewith.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____

John W. Bailey
Reg. No. 32,881

P. O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

JWB/KLR
Attachment: Version with Markings to Show Changes Made
Exhibits 1 and 2

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claims 2, 4, and 11 are canceled.

Claims 15-27 are added.

The following claims are amended:

3. (Twice Amended) [The] A mutant α-amylase [according to Claim 2,] obtained by making a substitution of [wherein] an amino terminal sequence from 1st Asp through 19th Gly of SEQ ID NO:1 or an amino terminal sequence corresponding to 1st Asp through 19th Gly of SEQ ID NO:1 of a sequence having at least 95% [70%] homology to SEQ ID NO:1, [is substituted] with an amino acid sequence from 1st His to 21st Gly of SEQ ID NO:2, [encoding another liquefying α-amylase]

wherein said mutant α-amylase possesses increased heat resistance and maintains resistance to chelating agents when compared to SEQ ID NO:1.

5. (Three Times Amended) A mutant α-amylase obtained by introducing a first mutation and a second mutation [two kinds of mutations] into SEQ ID NO:1 or an amino acid sequence having at least 95% [70%] homology to SEQ ID NO:1,

wherein said [a] first mutation consists of [is] a substitution or a deletion of at least one amino acid residue selected from the group consisting of the 11th Tyr, 16th Glu, 49th Asn, 84th Glu, 144th Ser, 167th Gln, 169th Tyr, 178th Ala, 188th Glu, 190th Asn, 205th His and 209th Gln, and

wherein said [a] second mutation consists of [is] a substitution of a sequence corresponding to the 11th to 100th [11 to 100] amino acid residue [residues] from the amino terminus of the amino acid sequence set forth in SEQ ID NO:1, and

wherein said mutant α-amylase possesses increased heat resistance and maintains resistance to chelating agents when compared to SEQ ID NO:1.

6. (Twice Amended) The mutant α-amylase according to Claim 5, wherein said first mutation consists of [comprises]:

the substitution of an amino acid residue selected from the group consisting of: the 11th Tyr of SEQ ID NO:1 with Phe, the 16th Glu of SEQ ID NO:1 with Pro, the 49th Asn of SEQ ID NO:1 with Ser, the 167 Gln of SEQ ID NO:1 with Glu, the 169th Tyr of SEQ ID NO:1 with Lys, the 190th Asn of SEQ ID NO:1 with Phe, the 205th His of SEQ ID NO:1 with Arg, and the 209th Gln of SEQ ID NO:1 with Val,

and wherein said second mutation consists of [comprises]:

substituting an amino terminal sequence from 1st Asp through 19th Gly of SEQ ID NO:1 with an amino acid sequence from 1st His to 21st Gly of SEQ ID NO:2.

13. (Amended) A mutant α-amylase obtained by making a substitution or deletion of at least one amino acid residue of specific positions in SEQ ID NO:1, or by making a substitution or deletion of at least one amino acid residue corresponding to the above-mentioned amino acid residue in a sequence having at least 95% homology to SEQ ID NO:1,

wherein said at least one amino acid residue is selected from the group consisting of:

the 11th Tyr, 16th Glu, 49th Asn, 84th Glu, 144th Ser, 167th Gln, 169th Tyr, 178th Ala, 188th Glu, 190th Asn, 205th His and 209th Gln, and

wherein said mutant α-amylase:

(i) decomposes α-1,4-glycoside bonds of starch, amylose, amylopectin, and partially decomposed products thereof;

(ii) produces glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose from amylose;

(iii) does not act on pullulan;

(iv) exhibits a residual activity of at least 70% in a pH range of 6.5 to 11 under treatment conditions of 40°C and 30 minutes;

(v) acts in a temperature range of 20°C to 80°C;

(vi) exhibits a residual activity of at least 80% when incubated at 40°C, or at least 60% when incubated at 45°C, for 30 minutes in 50 mM glycine-sodium hydroxide buffer at pH 10;

(vii) has a molecular weight of 55,000 ± 5,000 as measured by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis;

(viii) has an isoelectric point of about 4.2 as measured by isoelectric focusing;

(ix) has a residual activity of at least 90% when treated at pH 10 and 30°C for 30 minutes in a 0.1% solution of a surfactant selected from the group consisting of:

sodium linear alkylbenzenesulfonates, sodium alkylsulfates, sodium polyoxyethylene alkylsulfates, sodium α-olefinsulfonates, sodium salts of α-sulfonated fatty acid esters, sodium alkylsulfonates, SDS, soap, and Softanol;

(x) is inhibited by 1 mM Mn^{2+} by about 75%, or by 1 mM Sr^{2+} or 1 mM Cd^{2+} by about 30 to 40%, when treated at pH 10 and 30°C for 30 minutes; and

(xii) comprises an amino acid sequence which is at least [95%] 70% homologous to SEQ ID NO:1.

SECOND SITE: http://www.ualberta.ca/~stothard/javascript/index.html

The Sequence Manipulation Suite: Identity

SEQ ID NO 1 vs SEQ ID NO 4
Alignment length (not including identical gaps): **480**
Identical residues: **463**
Similar residues: **4**
Percent identity: **96.5%**
Percent similarity: **97.3%**

Comparison of:
(A) 80655961 >_ **SEQ ID NO 1** - 480 aa
(B) 80655962 >_ **SEQ ID NO 2** - 485 aa
using matrix file: BLOSUM50, gap penalties: -14/-4

66.9% identity in 483 aa overlap; score: 2379

```
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       .: ::::::::.:::: :::.:::::::.:::: :.. ::::.:::::.::.:: ::::::::
_      NGTNGTMMQYFEWHLPNDGNHWNRLRDDAANLKSKGITAVWIPPAWKGTSQNDVGYGAYD
             10         20         30         40         50         60

               70         80         90        100        110        120
_      LYDLGEFNQKGTVRTKYGTKAQLERAIGSLKSNDINVYGDVVMNHKMGADFTEAVQAVQV
       ::::::::::::::::::::.::. :. ::::.: :.:::::::::: ::: :: :.::.:
_      LYDLGEFNQKGTVRTKYGTRSQLQGAVTSLKNNGIQVYGDVVMNHKGGADGTEMVNAVEV
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              130        140        150        160        170
_      NPTNRWQDISGAYTIDAWTGFDFSGRNNAYSDFKWRWFHFNGVDWDQRYQ-ENHIFRFAN
       : .:: :.:::: :::.::: ::: ::.:.:.:::::.::.:.::::  : .:.:..: .
_      NRSNRNQEISGEYTIEAWTKFDFPGRGNTHSNFKWRWYHFDGTDWDQSRQLQNKIYKFRG
            130        140        150        160        170        180

     180        190        200        210        220        230
_      TN--WNWRVDEENGNYDYLLGSNIDFSHPEVQDELKDWGSWFTDELDLDGYRLDAIKHIP
       :.  :.:.:: ::::::::. ..:::.::: .:::.:: :.:. :.::::.:.::.::
_      TGKAWDWEVDIENGNYDYLMYADIDMDHPEVINELRNWGVWYTNTLNLDGFRIDAVKHIK
            190        200        210        220        230        240

       240        250        260        270        280        290
_      FWYTSDWVRHQRNEADQDLFVVGEYWKDDVGALEFYLDEMNWEMSLFDVPLNYNFYRASQ
       . :: ::. : :: . . .:.:.:.:.:.:.:: ::.. .:. :.::::::.::.: ::.
_      YSYTRDWLTHVRNTTGKPMFAVAEFWKNDLAAIENYLNKTSWNHSVFDVPLHYNLYNASN
            250        260        270        280        290        300

       300        310        320        330        340        350
_      QGGSYDMRNILRGSLVEAHPMHAVTFVDNHDTQPGESLESWVADWFKPLAYATILTREGG
       .:: .:::::: ::.:. ::.:::::::::::.::::.::.: .:::::::: ::::: :
_      SGGYFDMRNILNGSVVQKHPIHAVTFVDNHDSQPGEALESFVQSWFKPLAYALILTREQG
            310        320        330        340        350        360
```

```
        360       370       380       390       400       410
_       YPNVFYGDYYGIPNDNISAKKDMIDELLDARQNYAYGTQHDYFDHWDVVGWTREGSSSRP
        ::.:::::::::::. .. . :. :: ::.:::.:::::::::::::: :..:::::.::.:
_       YPSVFYGDYYGIPTHGVPSMKSKIDPLLQARQTYAYGTQHDYFDHHDIIGWTREGDSSHP
             370       380       390       400       410       420

        420       430       440       450       460       470
_       NSGLATIMSNGPGGSKWMYVGRQNAGQTWTDLTGNNGASVTINGDGWGEFFTNGGSVSVY
        ::::::::::.:::::.:::::::..::.: :.::: ..:::::.:::::.: .:::.::::.
_       NSGLATIMSDGPGGNKWMYVGKHKAGQVWRDITGNRSGTVTINADGWGNFTVNGGAVSVW
             430       440       450       460       470       480

        480
_       VNQ
        :.:
_       VKQ
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EVNRSNRNQEISGEYTIEAWTKFDFPGRGNTHSNFKWRWYHFDGTDWDQSRQLQNKIYKF
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IKYSYTRDWLTHVRNTTGKPMFAVAEFWKNDLAAIENYLNKTSWNHSVFDVPLHYNLYNA
SNSGGYFDMRNILNGSVVQKHPIHAVTFVDNHDSQPGEALESFVQSWFKPLAYALILTRE
QGYPSVFYGDYYGIPTHGVPSMKSKIDPLLQARQTYAYGTQHDYFDHHDIIGWTREGDSS
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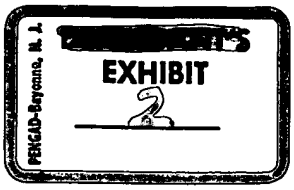

Comparison of:
(A) 77810651 >_ SEQ ID NO: 1 - 480 aa
(B) 77810652 >_ SEQ ID NO: 4 - 480 aa
using matrix file: BLOSUM50, gap penalties: -14/-4

96.5% identity in 480 aa overlap; score: 3335

```
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_    :::::::::::::::::::::::::::: ::::.:::::::::::::::::::::::::
     DGLNGTMMQYYEWHLENDGQHWNRLHDDAEALSNAGITAIWIPPAYKGNSQADVGYGAYD
          10        20        30        40        50        60

          70        80        90       100       110       120
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          70        80        90       100       110       120

         130       140       150       160       170       180
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     NPSNRWQDISGVYTIDAWTGFDFPGRNNAYSDFKWRWFHFNGVDWDQRYQENHLFRFANT
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         190       200       210       220       230       240
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_    :::::::::::::::::::::::::::.::::::::::::::::::::::::::::::::
     NWNWRVDEENGNYDYLLGSNIDFSHPEVQEELKDWGSWFTDELDLDGYRLDAIKHIPFWY
         190       200       210       220       230       240

         250       260       270       280       290       300
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         310       320       330       340       350       360
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_    ::::::::::::::::.::::::::::::::::::::::::::::::::::::::::::::
     SYDMRNILRGSLVEAHPIHAVTFVDNHDTQPGESLESWVADWFKPLAYATILTREGGYPN
         310       320       330       340       350       360

         370       380       390       400       410       420
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_    ::::::::::::::::::::::::::::::::::::::::::::::.::::::.::::::::
     VFYGDYYGIPNDNISAKKDMIDELLDARQNYAYGTQHDYFDHWDIVGWTREGTSSRPNSG
         370       380       390       400       410       420

         430       440       450       460       470       480
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_    ::::::::::::::::::.:.::::::::::.:::::::::::::::::::::::::::::
     LATIMSNGPGGSKWMYVGQQHAGQTWTDLTGNHAASVTINGDGWGEFFTNGGSVSVYVNQ
         430       440       450       460       470       480
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done at: http://vega.igh.cnrs.fr/bin/lalign-guess.cgi